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PROJECT TITLE : Tobacco Physiology and Biochemistry
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LOW NICOTINE STUDY

A. Objective: To investigate the biochemistry of the nicotine biosynthetic pathway at the putrescine N-methyltransferase (PMT) step and specifically to isolate PMT from tobacco root extracts.

B. Results: Ammonium sulfate samples prepared from the roots of hydroponically grown burley 21 tobacco plants were fractionated on two 500 ml bed volume phenyl-Sepharose columns. PMT activities eluted off these columns were similar to the activities obtained from roots of plants from groups 25 and 26. The specific activity for the peak PMT samples was determined to be 88.9 units (1). Phenyl-Sepharose purified material was further processed by DEAE/AHS chromatography. Various low activity DEAE/AHS fractions from all previous harvests were pooled to obtain batch 5C (2).

Alkaline phosphatase (AP) was renatured according to the method developed by Delvaux et al. (3). However attempts to renature PMT have been unsuccessful to date (2).

Mouse anti-PMT serum was received from the contract lab (Pocono Lab, PA). This was the first bleeding since the animals were challenged with PMT. ELISA screening for the presence of the PMT antibody yielded positive results for sera from two mice immunized with putative PMT. Protein blots made from SDS-PAGE of batch 1 PMT were examined by antibody. The putative PMT protein band at 60 kD showed a strong positive reaction as visualized for the presence of primary antibody using an alkaline phosphatase secondary antibody conjugate (4).

The batch PMT material was resolved by SDS-PAGE and electroblotted. The PMT band was digested by cyanogen bromide (CNBr) (5,6) and sequenced by a vendor. The CNBr digest of the putative PMT band was resolved into a number of peaks by reverse phase HPLC (5).

Amplification of PR17 cDNA insert was attempted using T3 and T7 primers. A fragment of DNA of appropriate size was amplified (7). Cloning of the PR17 insert into vector pBI121 was also tried. The results are being evaluated (7). The degenerate oligonucleotide probes for putative PMT gene were labeled and hybridized to northern blots of poly A+ RNA prepared from tobacco roots. The results were inconclusive (8). Several cDNA clones (pVM1, pVM2, pVM3, PR7, PR12, PR19 and PR50) have been sequenced by using automated DNA sequencer and T3 dye-labeled primer (9).

C. Plans: Harvest group 28 plants, and process the tobacco roots to the 40-65% ammonium sulfate stage. Continue to fractionate ammonium sulfate extracts by chromatographic methods. Continue efforts to renature PMT. Continue to sequence CNBr generated fragments of the putative PMT protein. Continue to sequence selected cDNA clones.

Attempt to amplify PMT-like sequences using degenerate primers.

D. References:

1. Lyle, J. Notebook No. 8856, p. 200.

2. Turner, D. Notebook No. 8973, p. 200.

3. Delvaux, A.; Lemos, M.; Moreau, C.; Erneaux, C. Regeneration of enzymatic activity after sodium dodecylsulfate/polyacrylamide gel electrophoresis and zinc acetate staining: The example of inositol 1,4,5-triphosphate 5-phosphatase. Analytical Biochemistry 188: 219-221 (1990).

4. Yu, T. Notebook No. 9002, p. 82.

5. Nakatani, H. Notebook No. 8384, p. 170.

6. Bower, P. Notebook No. 9032, pp. 16-17, 22-25.

7. Malik, V. Notebook No. 8974, p. 78.

8. Wahab, S. Notebook No. 8983, pp. 170-171.

9. Michalik, T. Notebook No. 9036, p. 77.